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Water uptake on polar stationary phases under conditions for hydrophilic interaction chromatography and its relation to solute retention

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ABSTRACT

Since water associated with the stationary phase surface appears to be the essence of the retention mechanism in hydrophilic interaction chromatography (HILIC), we developed a method to characterize the water-absorbing capabilities of twelve different HILIC stationary phases. Adsorption isotherms for non-modified and monomerically functionalized silica phases adhered to a pattern of monolayer formation followed by multilayer adsorption, whereas water uptake on polymerically functionalized silica stationary phases showed the characteristics of formation and swelling of hydrogels. Water accumulation was affected by adding ammonium acetate as buffer electrolyte and by replacing 5% of the acetonitrile with tertiary solvents capable of hydrogen bonding such as methanol or tetrahydrofuran. The relationship between water uptake and retention mechanism was investigated by studying the correlations between retention factors of neutral analytes and the phase ratios of HILIC columns, calculated either from the surface area (adsorption) or the volume of the water layer enriched from the acetonitrile/water eluent (partitioning). These studies made it evident that adsorption and partitioning actually coexist as retention promoters for neutral solutes in the water concentration regime normally encountered in HILIC. Which factors that dominates is dependent on the nature of the solute, the stationary phase, and the eluting conditions.

1. Introduction

The interest in hydrophilic interaction chromatography (HILIC) has increased substantially over the last decade. The main reasons for this surge are that HILIC can provide retention for polar compounds lacking retention in reversed phase HPLC, and that the partly aqueous eluents high in acetonitrile that are typically used are almost ideally suited for coupling to electrospray mass spectrometry. HILIC has therefore been successfully applied to separation of a variety of polar compound including peptides, proteins, carbohydrates, oligosaccharides, drugs, metabolites, and various natural compounds [1–4].

Yet, the retention mechanism in HILIC is still under debate, although it is commonly believed that the retention in HILIC is driven mainly by the partitioning of solutes between the bulk mobile phase and a water enriched layer that is semi-immobilized on HILIC stationary phases, as postulated by Alpert in his seminal 1990 paper on HILIC [5]. His assumption was deduced from previous works dealing with separation of carbohydrates on polar phases [6–8] and has been corroborated by many later studies [9–13]. However, those studies have also pointed out that the functional groups on the phase surface contribute to the selectivity in HILIC *via* hydrogen bonding and dipole-dipole interactions. Also important are electrostatic interactions between charged solutes and hydrophilic stationary phases due to the presence of ionic groups incorporated intentionally or inadvertently, as per ionized silanol groups on silica-based stationary phase surfaces. Residual silanol groups may contribute to cation exchange and repulsion of anions, interactions that would be superimposed on partitioning as retention mechanism.

Separations in HILIC are furthermore affected by type and amount of electrolytes (buffer salts) added to the eluent [14], an addition that is required to reach stable retention – in particular for solutes that are charged or capable of being protonated or dissociated in the eluent. The second reason for adding electrolytes is to shield residual silanol groups that would otherwise lead to electrostatic interaction on silica-based stationary phases [12,14]. To further complicate the issue, solutes interacting with the water enriched layer are faced with a compositional gradient of solvent components and electrolytes. This gradient extends from the bulk

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eluent to the inner surface layer, which in the case of a solid and impervious material such as silica will cause a significant perturbation of the liquid structure of the adsorbed water, extending several molecular layers from the surface [15,16]. This layer will normally also be enriched in electrolytes, since ions from buffer compounds typically used in HILIC are better solvated in solvents with high dielectric constants such as water. Many factors thus contribute to a complex retention mechanism in HILIC and studies on the water-enriched layer are therefore crucial to gain a better understanding of the retention patterns of various kinds of solutes in HILIC.

There are currently numerous commercially available HILIC columns with a wide variety of functional groups, such as underivatized silica or silicas functionalized with low molecular weight 2,3-dihydroxypropyl (diol), 3-aminopropyl, amide, cyclodextrin, sulfoalkylbetaine, or phosphocholine as ligands that are grafted onto surface silanols, as well as polymer-coated silicas featuring interactive layers consisting of 2-hydroxy- or 2-sulfoethylated poly(aspartamide), sulfobetaine and phosphocholine methacrylates, and sulfonated polystyrene-divinylbenzene resin [4,13]. New HILIC stationary phases are also continuously being developed and investigated to further develop the HILIC mode as a liquid chromatographic technique that offers new selectivity dimensions that are largely complementary to reversed phase HPLC [17–20].

One of the prerequisites for a stationary phase to be useful in HILIC is (per definition) its water uptake capacity, which seems to vary substantially among different materials and appears to be a function of their structure and functionality [21–23]. The present study was therefore devised to shed some light on ways in which water is taken up by hydrophilic separation materials with different functionalities and characteristics and how this correlates to their retention and separation mechanisms. Since water associated with the stationary phase surface appears to be the essence of the retention mechanism in HILIC, we wanted to find a universal and direct method to characterize the water-absorbing capacities of HILIC stationary phases. We were also curious to investigate the influence of different surface functional groups in formation of the water enriched layer. Therefore, the practical part of this work was centered on determining the water uptake capabilities of polar stationary phases using coulometric Karl Fischer titration, the technique most commonly used for accurate determination of water content at low levels. The secondary aim of the project was to elucidate how the water-retaining abilities were affected by admixture of water-soluble organic modifiers with hydrogen bonding capabilities, such as methanol (MeOH) and tetrahydrofuran (THF), to eluents consisting of water and acetonitrile only. We further wanted to study the effects of varying the concentration of ammonium acetate as buffer salt in the mobile phase in terms of the water uptake capacities at a fixed water:acetonitrile ratio in the mobile phase. The final aim was to investigate HILIC at the level of first principles, *i.e.*, to clarify if correlations exist between the level of water uptake and the retention of a select set of neutral polar solutes on several HILIC phases, and from this draw conclusions on the role of the water-enriched layer in the retention mechanism. Preliminary data and results from this study have partially been disclosed in posters and talks at recent scientific conferences [24,25].

2. Experimental

2.1. Chemicals

Ammonium acetate (≥98%) was purchased from Scharlau Chemie (Barcelona, Spain). Hydranal-Coulomat AG-Oven reagent, cytidine (≥99%), and uridine (≥99%) were from Fluka (Buchs, Switzerland). The HPLC grade solvents acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), and toluene were purchased from Fischer Chemicals (Loughborough, UK). Methanol (MeOH), and tetrahydrofuran (THF) were from JT Baker (Deventer, The Netherlands), and BDH (Poole, UK), respectively. Uracil (≥99%), adenosine (≥99%), cytosine (≥99%), methyl glycolate (98%) (M-GLY), α-hydroxy-γ-butyrolactone (99%) (HBL), thymine (≥99%), and thymidine (≥99%) were from Aldrich (Steinheim, Germany). Adenine and 1,3-dihydroxyacetone (DHA) were obtained from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm^{-1}) was supplied by a Merck Millipore (Billingham, MA) Ultra-Q water purification system.

2.2. Instrumentation

An autosampling coulometric Karl Fischer titration system was prepared by connecting an HP 1050 HPLC autosampler (Agilent Technologies, Santa Clara, CA, USA) with a 756 KF coulometer (Metrohm, Herisau, Switzerland) for determination of water. An FMI model RP-G50 lab pump (Fluid Metering Inc, Syosset, NY, USA) was used to pump Hydranal-Coulomat AG-Oven reagent from the titration cell to the autosampler inlet at a flow rate of ≈1.5 ml/min. The autosampler outlet was connected back to the titration cell to form a closed system. Thereby the Hydranal reagent acted as carrier solution, conveying the samples from the autosampler to the titration cell. A signal cable was connected between autosampler and titrator to synchronize the "Start" and "Ready" signals. The titration data were acquired and processed by VESUV 2.0 software from Metrohm using an Intel-based personal computer. A Mettler AT 250 balance (Mettler-Toledo, Greifensee, Switzerland) with 0.1 mg resolution was used to weigh the samples for titration and a Sartorius BP 310S (Sartorius, Göttingen, Germany) balance with resolution of 1 mg was employed to weigh the columns for determination of their total mobile phase volume. An HP 1050 HPLC system (Agilent Technologies), consisting of a quaternary eluent pump, an autosampler, and a diode array detector, was employed for chromatographic testing of the HILIC stationary phases. ChemStation A10.01 software from Agilent Technologies was used to control the HPLC system and to acquire the chromatographic data.

2.3. Sample preparation

Fourteen solutions representing a variety of mobile phase compositions applicable in HILIC were prepared according to the listing in Table 1. Dried acetonitrile having a water content of around 70 ppm was used for preparation of those mobile phases. The water uptake analyses were made on twelve different commercial HILIC stationary phases. Seven samples of each type of stationary phase were prepared in 2 mL glass vials by accurately weighing in around 150 mg and immediately thereafter sealing the vials with PTFE-lined septum caps. Each vial was thereafter punctured with a 0.4 mm hypodermic needle and placed inside a thermostatted vacuum oven at ≈100 Pa pressure and 90 °C for around three days to dry. After drying, the needles were pulled and the vials were again weighed to calculate the loss of water in each sample. The dry weights of HILIC phases in the vials were thereby deduced. Thereafter, accurate volumes of the first seven mobile phases listed in Table 1 were added, one to each vial, by syringe transfer. Equilibrium was established by swirling the vials on an orbital shaker for 1 h at room temperature (21–24 °C). The vials were then centrifuged at 5400 rpm for 5 min to settle the particles as pellets at the bottom of the vials. The supernatants from each sample vial were thereafter subjected to automated coulometric Karl Fischer titration to determine the amount of water in the bulk mobile phase. After the titrations were completed, the samples were decanted and dried again according to the procedure described above, whereafter each of them was equilibrated with one of the seven remaining mobile phases (8–14) for 1 h, centrifuged, and the